# DON (Vomitoxin) Handbook

**United States Department of Agriculture**

Grain Inspection, Packers and Stockyards Administration

Federal Grain Inspection Service

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United States Department of Agriculture
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# Program Handbook

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## DON (Vomitoxin) Testing

Foreword

The DON (Vomitoxin) Handbook has been established to consolidate instructions for performing DON testing. This handbook illustrates step-by-step procedures for testing and certifying grain for DON.

All official inspection personnel authorized or licensed to perform DON testing shall reference this handbook for procedures.

This handbook supersedes FGIS Directive 9180.57, "DON (Vomitoxin) Testing Service", dated, 4-3-00.

***/s/ John Sharpe***

David Orr, Director
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U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
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# DON (Vomitoxin) Testing

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# CHAPTER 1

# GENERAL INFORMATION

## 1.1 PURPOSE

This directive establishes official procedures for determining Deoxynivalenol (DON) in grain and certifying the official results. This service is provided as official criteria under the authority of the United States Grain Standards Act (USGSA), as amended.

## 1.2 BACKGROUND

DON, also referred to as vomitoxin, is a naturally occurring mycotoxin produced by several species of Fusarium. Wet and cool weather from flowering time on to maturity promotes infection, resulting in scab or head blight in barley, wheat, oats, and rye.

The Federal Grain Inspection Service (FGIS) of the Grain Inspection, Packers and Stockyards Administration (GIPSA) provides DON testing service as official criteria for wheat, barley, oats, and corn. All official DON testing of grain is performed as prescribed in this directive by authorized employees of FGIS or licensed delegated/designated agency personnel.

Individuals wanting grains officially tested for DON should contact the nearest FGIS field office or delegated/designated agency.

DON test results are not reported to the Food and Drug Administration (FDA) because action limits are not established at this time.

## 1.3 DISCLAIMER CLAUSE

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

## 1.4 APPROVED TEST METHODS

The methods listed below have been conformance tested to perform within FGIS specifications. Each of the approved test methods has been certified to provide results accurate up to the conformance test level at which they were approved. Any test results that are above the conformance limits are reported as exceeding the established limit unless a supplemental analysis is performed.

| FGIS APPROVED TEST METHODS | | | |
|---|---|---|---|
| Method and Test Kit | Approved for | | Conformance Limit(s) |
| | Qualitative | Quantitative | |
| Myco✔ DON (Strategic Diagnostics Inc.) | | X | 3 PPM |
| RIDASCREEN®FAST DON (r-Biopharm) | | X | 5 PPM |
| AgriScreen (Neogen) | X | X | 5 PPM (quantitative test) |
| Veratox (Neogen) | X | X | 5 PPM (quantitative test) |
| Fluoroquant (Romer) | | X | 5 PPM |
| AccuTox™ (Romer) | | X | 5 PPM |
| EZ-Quant DON (Diagnostix) | | X | 5 PPM* |
| EZ-Quant DON 0.5 ppm (Diagnostix) | | X | 2.5 PPM* |
| Veratox 5/5 (Neogen) | | X | 5 PPM** |
| DON FQ (Vicam) | | X | 5 PPM |

* The EZ-Quant DON Test kit (part number 600312) for barley, malted barley, wheat, and corn is approved to a conformance limit of 5 ppm. The EZ-Quant DON 0.5 ppm test kit (part number 600313) is exclusively for barley and malted barley and is approved to a conformance limit of 2.5 PPM.

** The Veratox 5/5 test kit is approved to a conformance limit of 5 ppm. Using the optional extraction procedure limits the conformance level to 2.5 ppm.

The following chart lists the DON field test kits and the grains/commodities for which they have been approved. For information concerning the testing of other grains/commodities, contact the Policies and Procedures Branch.

| Test Method | Grain/Commodity | | | | | | |
|---|---|---|---|---|---|---|---|
| | Barley | Malted Barley | Corn | Oats | Wheat | Wheat Flour | Wheat Midds |
| DON Fluoroquant | X | X | X | X | X | | |
| DON AccuTox | X | X | X | X | X | X | X |
| Veratox | X | X | X | X | X | | |
| Veratox 5/5 | X | X | X | X | X | | |
| AgriScreen | X | X | X | X | X | | |
| EZ-Quant DON (0.5 -5.0) | X | X | X | | X | | |
| EZ-Quant DON (0.5-2.5) | X | X | | | | | |
| RIDASCREEN® FAST DON | X | X | X | X | X | | |
| Myco✔DON | X | X | X | X | X | | |
| DON FQ | X | X | X | X | X | | |

## 1.5 TESTING SERVICES

Applicants requesting DON testing must specify whether qualitative or quantitative testing service is desired. If qualitative analysis is requested, the applicant must specify the level desired (e.g., 1, 2 ppm). Three types of DON testing services are available as follows:

a. Submitted Sample Service.

Analysis based on a sample submitted by the applicant for service.

b. Official Sample-Lot Service.

Analysis based on an official sample obtained and analyzed by official personnel.

(1) Single lot inspection.

Samples may be obtained and tested on either an individual carrier basis or a composite sample basis (maximum of five railcars or fifteen trucks per composite sample).

(2) Unit train inspection under the CuSum Loading Plan.

Unit trains are analyzed on a sublot basis for wheat and barley and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

For unit trains, the sublot size for DON testing and for grade analysis may be different. For example, an applicant may request grade analysis on the basis of a sublot containing two cars and request DON analysis on the basis of five cars.

The maximum size sublot for DON testing is five railcars for unit trains consisting of less than 200,000 bushels, or less than 50 cars. For unit trains consisting of 200,00 bushels or more, or 50 railcars or more, the maximum sublot size is ten railcars.

(3) Export shiplots

Export shiplots are analyzed on a sublot basis for wheat and barley and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

The testing frequency for shiplot grain will be the same as the sample for grade analysis unless the applicant specifically requests DON analysis on the basis of a component sample.

(4) Supplemental Testing.

Upon request, supplemental testing may be performed as follows:

Composite samples may be analyzed in addition to the sublot test for wheat and barley shiplots or unit trains.

(5) Alternate Testing.

Upon request, alternate testing methods may be used, provided that the minimum testing requirements are met. Examples of alternate testing include:

(a) Sublot testing may be used instead of composite sample analysis for grains routinely tested on a composite basis.

(b) Grain shipments may be tested on a component sample basis in lieu of the sublot basis under the provisions of Book III, Inspection Procedures. Components are combined and averaged to determine the sublot result. Component samples will not be designated as a material portions due to DON because the FDA has not established action limits at this time. Acceptable quality will be based on the sublot result as compared to the contracted "maximum" specification.

c. Warehouse Sample-Lot Inspection Service.

Analysis based on an official sample obtained by a licensed warehouse sampler and analyzed by official personnel.

## 1.6 REVIEW INSPECTIONS

Sections 800.125 and 800.135 of the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factor and official criteria may be handled separately even though both sets of results are reported on the same certificate.

Review inspection services for DON are provided on either a new sample or the file sample in accordance with the regulations. Board appeal inspection services are limited to the analysis of file samples.

Only one field review (reinspection or appeal inspection) is permitted for shiplot, unit train, or lash barge material portions when testing is performed on a sublot basis. However, if the applicant requests a review of the entire lot, up to three review levels of service (reinspection, appeal, board appeal) may be obtained for each sublot included in the lot. Inspection results for each review level shall replace the previous inspection result.

a. Reinspection Service.

The laboratory providing original testing services also provides reinspection services. Applicants may request either qualitative or quantitative analysis unless the original test was quantitative. Then, only a quantitative analysis is available.

b. Appeal Inspection Service.

FGIS field offices provide appeal DON testing services. Field offices not equipped to provide testing will make arrangements with another FGIS office to provide the most timely service possible. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available.

If samples are sent to a field office for analysis, write the words **"DON APPEAL"** in the “Remarks” section of the grain sample ticket and on the back of the mailing tag.

c. Board Appeal Inspection Services.

Board appeal inspection services are limited to the file sample and are provided by the Board of Appeals and Review (BAR) in Kansas City. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available. The High Performance Liquid Chromatography (HPLC) method is also available for determining DON in Board appeal samples. The applicant must specify the HPLC method as the desired determination method. Otherwise, the Board appeal inspection will be conducted using the rapid method (test kits).

When sending samples to the BAR, write the words **"DON BOARD APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

## 1.7 QUALITY ASSURANCE PROGRAM

The Technical Services Division (TSD), located at the Kansas City Technical Center, conducts a DON check sample program for all specified service points and laboratories providing testing services. TSD is responsible for preparing and distributing check samples each quarter to all official DON testing locations, analyzing check sample results, notifying field locations of any results indicating problems, and releasing a quarterly summary report to all participating laboratories. Field offices are responsible for routine supervision to assure all laboratories in their circuit provide accurate results. The TSD check sample program is designed to test the capability of the official system and to monitor the accuracy of approved testing methods. The check sample program provides limited performance information that can be used to supplement the routine supervision of official personnel performing testing services.

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# CHAPTER 2

# LABORATORY SAFETY

## 2.1 WORK AREA REQUIREMENTS

The work area requirements covered under this section apply to FGIS-occupied space only.

a. Sample Grinding Area.

Samples must be ground in space separate from the analytical space. The field office manager and safety officer must determine whether added ventilation or a dust removal device is needed in the grinding area to remove airborne dust particles. Refer to the FGIS Safety and Health Office in Washington, D.C. for assistance in determining whether added dust removal equipment (e.g., exhaust fan) is required.

b. Sample Testing Area.

Test methods that involve the use of volatile chemicals (e.g., acetonitrile, methanol) must be performed in FGIS-approved laboratory space. Testing methods that are free from hazardous materials may be performed (upon approval of the field office manager) in alternate locations. The field office manager and safety officer must evaluate the testing materials to determine if FGIS-approved laboratory space is required. If testing is performed in an alternate location (i.e., table-top in inspection lab), consideration must be given to lighting, plumbing, electrical, and ventilation requirements. Refer to the FGIS Safety and Health Office in Washington, D.C. for assistance in determining if FGIS-approved laboratory space is required and whether added ventilation (e.g., exhaust fan) is required for alternate testing locations.

## 2.2 FGIS LABORATORY REQUIREMENTS

FGIS-approved laboratories are required for mycotoxin testing that involves the use of hazardous materials (e.g., flammable liquids). The requirements covered under this section apply to FGIS-occupied space that is dedicated for the sole function of mycotoxin testing.

Some DON testing methods require the use of flammable liquids and suspected carcinogens. The building owner (private or GSA) must permit the use of chemicals (e.g., acetonitrile, methanol) in space used by FGIS. FGIS will provide testing services onsite only in facilities that provide protection to FGIS personnel.

Individual elevators may provide two kinds of space for FGIS personnel to perform onsite DON testing. The space may be located (1) in a building along with other occupants, or (2) in a building devoted exclusively to laboratory space.

In either case, the plan for the intended laboratory space is subject to inspection and approval by FGIS prior to construction. The Safety and Health Office and field office manager will review proposed plans and suggest ways to comply with the requirements.

The following are minimum requirements for FGIS-occupied laboratory space.

a. Location.

Locate the laboratory at least 100 feet from the base of the elevator headhouse. This distance is subject to negotiation when the elevator uses exterior grain legs and/or inclined belts in lieu of interior grain legs or where the headhouse is equipped with blow-out panels or the headhouse consists of a lightly covered framework.

Laboratories must meet the following requirements when they are located in a building with other occupants:

(1) Isolate the laboratory from nonlaboratory occupants using a fire barrier having at least a 1-hour fire resistance.

(2) Provide a fire barrier consisting of floors, ceilings, and interior walls.

(3) Provide all passageways and other openings that lead to adjacent interior space with self-closing fire doors having a 1-hour fire resistance. Do not block these doors open.

(4) Separate the space from central heating, ventilation, and air-conditioning using automatic-closing fire dampers in the heating, ventilation, and air-conditioning ducts near the fire-barrier, or provide a separate heating, ventilation, and air-conditioning system in the laboratory.

b. Size.

Dedicate the space strictly for laboratory (chemical) work. Supply adequate space for chemical analysis (minimum of 100 square feet).

c. Electrical System.

Provide the laboratory space with electrical power and lighting meeting the standards of the National Electrical Code. Wiring suitable for Class I location is not required. A three-wire system consisting of an energized wire, a neutral wire, and a grounding conductor is satisfactory. Install overhead lighting fixtures through ceilings that serve as fire barriers. Fixtures suspended below such ceilings are acceptable.

d. Plumbing.

Provide the laboratory space with a basin having hot and cold potable water and a sewer connection.

e. Exhaust System.

The exhaust system must remove chemical vapors from the work area. Normal air conditioning and heating may provide adequate ventilation when performing testing procedures in a building devoted exclusively for laboratory space. Refer to the FGIS Safety and Health Office in Washington, D.C. for assistance in determining whether added ventilation, such as a fume hood, is needed. If needed, situate the laboratory space so that hoods are vented to the exterior of the building. Fume hood ventilation will require a 6 or 8 inch diameter opening, either vertically through the ceiling and roof or horizontally through an exterior wall. In some cases, a portable hood may be sufficient.

f. Eyewash and Safety Shower Station.

Provide the laboratory space with eyewash equipment (eyewash bottle or permanent faucet-mounted fixture). A permanent, faucet-mounted eyewash fixture is highly recommended. A safety shower station must be installed in laboratories where acetonitrile-based extraction solvent (Romer-Fluoroquant test method) is used.

g. Cautionary Markings.

(1) Provide signs for the laboratory door(s) as follows:

(a) "Biohazardous Material Present"

(b) "No Smoking, Eating, or Drinking"

(c) "Flammable Material Present"

(d) "Wear Safety Protection"

(e) "Admittance of Authorized Personnel Only"

(2) Provide signs for the refrigerator used for storing test kits, chemicals, or solutions, as follows:

(a) "Biohazardous Material Present"

(b) "No Food or Drink to be Stored in this Refrigerator"

For further information concerning the laboratory space requirements, contact the FGIS Safety and Health Office.

## 2.3 SAFETY

FGIS employees must comply with good practices to ensure a safe and efficient work environment. To accomplish this, include the following as part of an overall FGIS laboratory/testing area "Standard Operating Procedure" (SOP). Maintain the SOP, this handbook, and current Material Safety Data Sheets (MSDS) at each laboratory/testing location.

During onsite supervision at agency locations, FGIS employees must assess their personal safety requirements. If personal safety is questionable, FGIS employees must determine if personal protective equipment can be used to correct the safety deficiency at the testing location. If FGIS employees cannot utilize personal protective equipment to provide for a safe work environment, then onsite DON supervision must occur only when the testing area is considered safe.

Interested persons are restricted from entering the DON testing area during testing unless accompanied by official personnel and must observe the health and safety rules while in the area.

a. General Safety Practices.

(1) Table-Top Testing.

FGIS personnel must abide by the following safety practices when performing testing in an alternate location (e.g., table-top in inspection lab).

(a) Do not smoke, eat, drink, or chew gum or tobacco in the immediate testing area.

(b) Wash hands immediately before and after eating, drinking, and smoking.

(c) Wear the following protective equipment when testing is being performed: disposable, fire-retardant laboratory coat; disposable, impermeable gloves; safety glasses or splash goggles.

(d) Wear a FGIS-approved disposable mask and hair protection when exposed to airborne grain dust.

(e) Do not store food or drink in the refrigerator used for storing chemicals and solutions, and test kits.

(f) Do not store masks and hair protectors in the grinding area where they might become contaminated by the dust particles.

(2) Laboratory Testing.

FGIS personnel must abide by the following safety practices when performing testing in an FGIS-approved laboratory.

(a) Do not smoke, eat, drink, or chew gum or tobacco in the laboratory.

(b) Wash hands immediately before and after eating, drinking, and smoking.

(c) Wear the following protective equipment: disposable, fire-retardant laboratory coat; disposable, impermeable gloves; safety glasses or splash goggles.

(d) Wear a FGIS-approved disposable mask and hair protection when exposed to airborne grain dust.

(e) Do not wear contact lenses in the immediate testing area (if testing with acetonitrile).

(f) Do not store food or drink in the laboratory refrigerator used for storing chemicals, solutions, and test kits.

(g) Do not store masks and hair protectors in the grinding area where they might become contaminated by the dust particles.

(h) Label all bottles and containers according to the Hazard Communication Program and the Chemical Hygiene Plan. In addition, when preparing mixtures of solutions, securely apply a label with the name of the solution, the preparation date, and the preparer's initials written in permanent ink.

(i) Store equipment outside the fume hood in a manner that will not clutter bench tops or obstruct movement.

(j) Prepare all chemical solutions and perform chemical analyses under a working fume hood.

(k) Limit the total quantity of waste chemicals in the laboratory to one liquid gallon.

(l) Limit the total amount of flammable solvent (including waste) in the laboratory to two gallons.

(m) Maintain a current MSDS for each chemical in the laboratory. If each supply of chemicals received does not have an MSDS enclosed, contact the company and request one immediately.

(n) Store flammable solvents in an approved storage cabinet.

(o) Store waste chemicals (e.g., acetonitrile, methanol) in impermeable metal containers meeting Underwriters Laboratory approval for Class I liquids. The containers must be capable of maintaining a tight seal and must be labeled "Flammable" or "Biohazardous Material" or both, as applicable.

(p) Contact an Environmental Protection Agency (EPA)-approved or EPA-certified waste disposal company and make arrangements for removal of chemical wastes or provide other suitable waste disposal procedures consistent with existing laws that do not create a hazard to the community.

## 2.4 SANITATION REQUIREMENTS

The sanitation requirements for spillage, labware, and excess sample extract listed in this section are applicable to testing performed at an FGIS-approved laboratory or an alternate testing location (e.g., table-top in the inspection lab).

Official agencies must adhere to the requirements for cleaning labware and should follow procedures established in their area for the disposal of excess sample extract.

Perform the following procedures only while wearing disposable, impermeable gloves, chemical splash goggles, and a fire-retardant laboratory coat. If hands become contaminated, wash immediately with soap and water.

a. Spillage.

Clean areas and materials contaminated by any extraction solution spills. Wipe up the affected areas using an absorbent cloth or paper towels, then wash the area with a soap/water solution. Place cleaning materials in a plastic waste bag, close tightly, and discard in a dumpster or landfill disposal site.

b. Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

c. Excess Sample Extract.

The disposition of excess sample extracts and solutions varies with the testing methodologies. All sample extracts containing chemicals such as methanol and acetonitrile are treated as hazardous chemicals and are disposed of in the chemical waste container. Unused extracts consisting of water only or a water/salt solution may be disposed of by pouring down the drain. Refer to the appropriate testing procedures for specific waste disposal instructions.

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# CHAPTER 3

# SAMPLE PREPARATION

## 3.1 SAMPLE SIZE AND PREPARATION

A sample of approximately 200 grams, with dockage and stones removed, is required for the DON testing and file sample (100 grams work portion, 100 grams file portion). An additional sample may be required if subsequent review inspections are requested. A similar sample size is recommended for submitted samples.

Obtain samples according to the guidelines in the Grain Inspection Handbook, Book I, "Grain Sampling." From the 100-gram ground work portion, divide (using a Boerner divider) out a portion of 50 grams for DON testing and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram.

## 3.2 GRINDING SAMPLES

Grind approximately 100 grams (dockage and stone free) of grain using a Romer Mill-Model 2a, Udy Grinder, Perten Falling Number Mill, Bunn Commercial Coffee Grinder, or an equivalent device that meets FGIS' performance requirements.

**SAFETY NOTE: OPERATOR MUST OBSERVE SAFETY PRECAUTIONS AND WEAR EYE PROTECTION WHEN OPERATING THE GRINDER. SEE THE OPERATOR'S MANUAL FOR MORE SAFETY TIPS.**

The grinding apparatus must be adjusted to produce a particle size that is sufficiently fine enough to obtain a homogeneous blend. Generally, a sufficiently coarsely ground sample of wheat resembles whole wheat flour, while a sample that is too coarsely ground has the appearance of bulgur or semolina. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

a. <u>Procedures for Checking the Performance of the Grinder.</u>

To check the performance of equipment used for grinding **small grains (e.g., wheat and barley),** use the following procedures:

(1) Grind a sample portion of approximately 100 grams of relatively dry wheat (i.e., 13 percent or less moisture).

(2) Weigh the entire portion that was ground.

(3) Sieve the portion across a standard No. 20 wire woven sieve.

(4) Weigh the portion that passed through the sieve.

(5) Determine the percent of fine material, by weight, as follows:

Fines = weight from step (4) divided by the weight from step (2) X 100.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn having a moisture content of 14 percent or less.

b. Optimum Particle Size.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.

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# CHAPTER 4

# CERTIFICATION

## 4.1 BACKGROUND

Wheat, barley, corn, and oats are tested for DON under the authority of the United States Grain Standards Act (USGSA). Under the USGSA, DON results are recorded on the pan ticket, worksheet, or loading log and in the remarks section of the certificate.

Certify DON test results on grain in accordance with sections 800.160 through 800.166 of the regulations under the USGSA.

Upon the request of the applicant, separate certificates may be issued for grade and for DON when both are determined on the same lot.

Sections 800.125 and 800.135 of the regulations under the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately, even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria, and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

## 4.2 GENERAL PROCEDURES

The type of service requested and the test method used determine how DON results are recorded and certified.

a. Qualitative Testing.

(1) Record the results of a **qualitative service** on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

(2) If a **quantitative method** is used to provide qualitative service, record the test results on the work records in a quantitative measurement (e.g., 1.4 ppm) or a qualitative measurement (e.g., $\leq$ 2 ppm).

(3) Certify results as being equal to or less than a threshold.

b. Quantitative Testing.

(1) Test kits with conformance limit ending in .5 ppm (e.g., 2.5 ppm)

Record the results on the pan ticket and the inspection log to the tenth ppm.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Certify test results that are above the lower conformance level (e.g. 0.6 ppm) and below the upper conformance level (e.g., 2.4 ppm) to the nearest whole ppm. For example: A DON test result of 2.3 ppm obtained using a DON test kit with a conformance range of 0.5 - 2.5 ppm would result in the following certification statement "DON 2 ppm." Upon request of the applicant, the results may be certified to the tenth ppm.

Test results that are equal to the conformance limit are certified as equal to the conformance limit. For example: A DON test result of 2.5 ppm obtained using a DON test kit with a conformance range of 0.5 - 2.5 ppm would result in the following certification statement "DON 2.5 ppm."

Test results greater than the conformance limit are certified as exceeding the conformance limit. For example: A DON test result of 2.8 ppm obtained using a DON test kit with a conformance range of 0.5 - 2.5 ppm would result in the following certification statement "DON exceeds 2.5 ppm."

(2) Test kits with conformance limits rounded to the whole ppm (e.g., 5 ppm)

Record the results on the pan ticket and the inspection log to the tenth ppm.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Certify test results that are between 0.6 ppm and the conformance limit (e.g., 5 ppm) to the nearest whole ppm. For example: A DON test result of 5.4 ppm obtained using a DON test kit with a conformance range of 0.5 - 5 ppm would result in the following certification statement "DON 5 ppm." Upon request of the applicant, results between 0.6 ppm and the conformance limit (e.g., 5 ppm) may be certified to the tenth ppm.

Test results greater than the conformance limit are certified as exceeding the conformance limit. For example: A DON test result of 5.5 ppm obtained using a DON test kit with a conformance range of 0.5 - 5 ppm would result in the following certification statement "DON exceeds 5 ppm."

STANDARD REPORTING - QUANTITATIVE TESTING

| Test Kit Range | Test Result | Certify as: | Test Result | Certify as: | Test Result | Certify as: | Test Result | Certify as: |
|---|---|---|---|---|---|---|---|---|
| 0.5 - 2.5 ppm | 0.5 or less | ≤ 0.5 ppm | 0.6 - 2.4 | Nearest whole ppm | 2.5 ppm | = 2.5 ppm | 2.6 or more | > 2.5 ppm |
| 0.5 - 3 ppm | 0.5 or less | ≤ 0.5 ppm | 0.6 - 3.4 | Nearest whole ppm | * | * | 3.5 or more | > 3 ppm |
| 0.5 - 5 ppm | 0.5 or less | ≤ 0.5 ppm | 0.6 - 5.4 | Nearest whole ppm | * | * | 5.5 or more | > 5 ppm |

| OPTIONAL REPORTING - QUANTITATIVE TESTING | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Test Kit Range | | Test Result | Certify as: | | Test Result | Certify as: | | Test Result | Certify as: | | Test Result | Certify as: |
| 0.5 - 2.5 ppm | | 0.0 | Not Detected | | 0.6 - 2.5 | Actual tenth ppm | | * | * | | * | * |
| 0.5 - 3 ppm | | 0.0 | Not Detected | | 0.6 - 3.0 | Actual tenth ppm | | * | * | | * | * |
| 0.5 - 5 ppm | | 0.0 | Not Detected | | 0.6 - 5.0 | Actual tenth ppm | | * | * | | * | * |

## 4.3 CERTIFYING TEST RESULTS

a. Single lot inspection basis for trucks and railcars.

Certify each test result on a separate certificate.

b. Combined land carrier basis for trucks and railcars.

If an applicant requests DON testing on a composite basis (up to 5 railcars and 15 trucks) and the inspection for grade on the basis of individual carriers, factor only certificates are issued for the DON testing and separate grade certificates are issued for each carrier.

c. Composite Sample Testing for Shiplots.

Certify the composite results using the appropriate qualitative or quantitative statements.

d. Submitted Sample Testing.

Certify the results using the appropriate qualitative or quantitative statements.

e. Unit Train and Shiplot Inspection under the CuSum Loading Plan.

(1) Recording Test Results.

DON test results of sublot samples taken throughout loading are recorded on the loading log. A material portion occurs if the sublot result exceeds the limit as specified in the load order.

(2) Certifying Test Results.

When sublot samples are tested using a **quantitative method**, certify the lot based on the mathematical/weighted average (as applicable) of the accepted sublot results. When sublot samples are tested using a **qualitative method**, certify the lot results as equal to or less than the maximum limit (e.g., 2 ppm). If some sublots were reviewed using a quantitative method, continue to certify the lot as equal to or less than the maximum limit.

Certify material portions separately.

(3) Material Portions.

If a material portion occurs, the applicant has the option of requesting a review inspection. Review inspection results replace previous results when determining if a material portion exists.

If a material portion designation due to DON is not removed by the review inspection process, the applicant may leave the material portion on board and receive a separate certificate; return the grain to the elevator; or discharge the material portion along with additional grain in common stowage equivalent to one half the material portion quantity.

## 4.4 APPROVED STATEMENTS

Upon request of the applicant, the term vomitoxin may be used in lieu of the term DON in the certification statements.

a. Qualitative Service.

For qualitative service, certify results as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

"DON exceeds 2 ppm."

"DON equal to or less than 2 ppm."

b. Quantitative Service.

Use one of the applicable statements for certifying DON on a quantitative basis.

(1) When DON results are less than or equal to 0.5 ppm:

"DON equal to or less than 0.5 ppm."

(2) Certify DON test results between 0.6 ppm and the conformance limit (e.g., 5 ppm) to the nearest whole number in ppm.

"DON (result rounded to the nearest whole number) ppm."

**NOTE: The use of this statement is limited when testing with kits having a conformance limit ending in .5 ppm.**

(3) When quantitative test results are equal to the conformance limit (e.g., 2.5 ppm).

"DON (enter conformance limit) ppm."

**NOTE: The use of this statement is used only for test kits having a conformance limit ending in .5 ppm.**

(4) When quantitative test results are greater than the conformance limit (e.g., 2.5 ppm).

"DON exceeds (enter conformance limit) ppm."

(5) Board Appeals performed by the High Performance Liquid Chromatography (HPLC) method are certified to the tenth ppm.

"DON (record actual results to the nearest tenth) ppm. Results based on HPLC Reference Method."

c. Optional Quantitative Statements.

(1) At the request of the applicant, certify quantitative test results between 0.6 ppm and the conformance limit to the tenth ppm.

"DON (result in tenths) ppm."

(2) At the request of the applicant, use the following statement when DON is not detected using a quantitative method (0.0 ppm).

"DON not detected."

**NOTE: If sublot results are combined and averaged and the lot average is equal to 0.0 ppm, but an individual sublot result exceeds 0.0 ppm, then the statement "DON less than or equal to 0.5 ppm" must be used.**

d. Additional Statements.

The statements listed below may be used in addition to the applicable qualitative/quantitative statements.

(1) At the request of the applicant, convert and certify the ppm result to parts per billion (ppb) using an approved statement. To convert ppm to ppb, multiply the ppm result by 1000.

"(Actual ppm result) ppm is equivalent to (converted ppb results) ppb."

(2) At the request of the applicant, convert and certify results in milligrams per kilogram (mg/kg) or micrograms per kilogram (µg/kg). Use the following equivalents to determine mg/Kg or µg/kg:

ppm = mg/kg ppb = µg/kg

(3) When certifying multiple DON results on the same certificate and the results are based on different sample types the certificate must reflect the difference. As a guideline, the multiple results are shown as follows:

"Sublot sample results: DON equal to or less than (threshold) ppm."

"Composite sample result: DON (actual result) ppm."

(4) Use this statement when the applicant requests the type of test shown on the certificate:

"Results based on (indicate type of test used) method."

(5) Upon request of the applicant, one of the following statements may precede the applicable results statement when test results are equal to or less than the specified threshold.

"The DON result is negative." OR "Negative DON."

**NOTE: These certification statements may be modified as deemed necessary.**

e. Reinspection, Appeal, Board Appeal Certificates.

(1) Results are reported on the same kind of certificate issued for the original service and supersede the previously issued inspection certificate.

Enter the following statement on the reinspection/appeal/board appeal certificate:

"This certificate supersedes Certificate No. (number) dated (date)."

(2) The superseded certificate is null and void as of the date of the subsequent (reinspection/appeal/board appeal) certificate.

"The superseded certificate has not been surrendered."

(3) When a file sample is used, enter the following statement on the reinspection/appeal/board appeal certificate:

"Results based on file sample."

(4) When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using one of the following applicable statements:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of the original inspection service."

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/ reinspection/appeal inspection) service."

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# CHAPTER 5

## NEOGEN - AGRISCREEN and VERATOX DON TEST KITS

## 5.1 TESTING AREA

The extraction solution and other materials used in the AgriScreen, and Veratox test kits does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

## 5.2 EXTRACTION PROCEDURES

a. Barley, Corn, Oats, and Wheat.

(1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.

(2) Label the collection container with the sample identification.

(3) Thoroughly mix the ground sample and weigh a 50-gram portion.

(4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.

(5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.

(6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.

(7) Filter the extract by pouring at least 15 ml through the filter paper.

b. Malted Barley.

Follow step numbers 1-7 listed above, then pass 3 ml of the filtered extract through a Bond Elut SPE cartridge at a flow rate of 1 ml per minute.

## 5.3 PREPARATION OF SOLUTIONS

a. Conjugate.

(1) Open one of the conjugate bottles and remove the rubber stopper.

(2) Cut the tip off the enclosed squeeze tube and squeeze the tube contents into the bottle.

(3) Replace the stopper and swirl contents until the pellet has dissolved.

ALLOW THE REHYDRATED CONJUGATE SOLUTION TO SET FOR 1 HOUR PRIOR TO USE.

Use the contents of the bottle until empty (**once rehydrated, contents must be used within 3 weeks**).

KEEP REFRIGERATED WHEN NOT IN USE.

b. Substrate.

Substrate is pre-activated, ready for use, and should be stored in the dark. Remove only one vial of substrate at a time from the foil pouch prior to use.

c. Stopping Reagents and DON Control.

Open the stopping reagents and the DON control bottles and set aside. Swirl to mix prior to use.

## 5.4 QUALITATIVE (SCREENING) TESTING

a. Testing Procedures.

**NOTE: The AgriScreen kit is supplied with a 1 ppm control. Users must purchase another control to perform screening at a different level.**

(1) Remove the red-marked mixing well strip and break off the needed number of wells (one well for each sample and one well for control). Return the unused strip to the package.

**NOTE: Do not run more than six wells (five samples plus one control) at a time unless using a multichannel pipettor.**

(2) Remove the antibody-coated well strip and break off the same number of wells. Return the unused strip to the package and tightly close the package opening.

(3) Mark one end of the antibody-coated well strip with C for control so that you can identify the wells after washing.

(4) Firmly place a pipette tip on the pipettor and add 100 microliters (µl) of conjugate to each mixing well. Discard the tip.

**NOTE: 100 µl of liquid is the amount drawn into the pipette tip when the pipettor plunger is depressed and then released slowly.**

(5) Remove the stopper from the control bottle. Firmly place a new pipette tip on the pipettor and add 100µl of the control to the first mixing well. Thoroughly mix by depressing the plunger five times. Discard the tip. Replace the rubber stopper on control bottle.

(6) Firmly place a new pipette tip on the pipettor and add 100 µl from the sample collection tube to second well of the red-marked mixing strip. Thoroughly mix by depressing the plunger five times. Discard the tip.

(7) Repeat step (6) for each additional sample.

(8) Transfer 100 µl from each red-marked mixing well to the corresponding antibody-coated well. Use a new pipette tip for each well. Discard the red-marked wells.

(9) Mix by sliding the wells back and forth on a flat surface in a manner to ensure adequate mixing (10 to 20 seconds) without splashing reagents from wells. **Wait 10 minutes** (begin time after mixing).

(10) The initial reaction is now completed. Shake out the contents of antibody-coated wells.

(11) Using a wash bottle, fill each antibody-coated well with distilled/deionized water and shake out. Repeat five times. Remove all water droplets by turning the wells upside down and vigorously tapping wells on a paper towel.

(12) Firmly place a new pipette tip on the pipettor and add 100 µl of substrate to each antibody-coated well. Discard the tip.

(13) Mix as instructed in step (9) and **wait 10 minutes** (begin time after mixing).

(14) Firmly place a new pipette tip on the pipettor and add 100 µl of stop solution to each well. Discard the tip. Mix by tapping gently on the side of the antibody well strip.

(15) Visually determine the levels at 1 ppm or 2 ppm only, or read the results in the Microwell Model EL 301 Strip Reader.

b. Reading the Results.

(1) Make sure that the Microwell reader is on and allowed to warm up for a minimum of 15 minutes before using.

(2) Remove the sample carriage and hit "Enter."

(3) Insert the W2 filter (405 nm) and hit "Enter."

(4) Insert the W1 filter (650 nm) and hit "Enter."

(5) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(6) Load the antibody-coated wells into the sample carriage so that the control is in position A1.

(7) Load the sample carriage into the strip reader so that position A1 is under the reader.

(8) Hit "Read" and record the value obtained for A1 (the control).

(9) Slide the carriage to position A2 and hit "Read."

(10) If the value is **EQUAL TO** or **LARGER THAN** that recorded for A1, the sample is **LESS THAN** or **EQUAL TO** the control. If the value is **SMALLER THAN** that recorded for A1, the sample contains **MORE THAN** the control.

(11) Slide the carriage to the next sample and hit "Read."

(12) Repeat step (11) for each of the remaining samples.

c. Reporting and Certifying Test Results.

(1) Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

(2) Certify results as being equal to or less than a threshold. (See the Certification Chapter of this handbook for detailed procedures and statements).

## 5.5 QUANTITATIVE TESTING

a. Testing Procedures.

(1) Remove the red-marked mixing well strip and break off the number of wells needed (five wells for controls and one for each sample) up to a maximum of twelve. Mark one end of red-marked mixing well strip with a 0 (zero) for the blank and the other end with an S for samples so that you can identify the wells. Place the wells in the well holder.

(2) Remove an equal number of antibody-coated wells. Mark one end of strip with a 0 (zero) for the blank and the other end with an S for samples and place the strip in the well holder with the 0 (zero) marked end on the left.

(3) Mix each reagent by swirling the reagent bottle prior to use.

(4) Firmly place a pipette tip on the 100 µl pipettor and add 100 µl of conjugate to each mixing well. Discard the tip.

(5) Remove the cap from the 0 ppm control bottle. Firmly place a new pipette tip on the 100 µl pipettor and add 100 µl from the 0 ppm control bottle to the first (labeled 0 (zero)) mixing well. Discard the tip and replace the cap on the control bottle.

(6) Remove the cap from the 0.5 ppm control bottle. Firmly place a new pipette tip on the 100 µl pipettor and add 100 µl from the 0.5 ppm control bottle to the second mixing well. Discard the tip and replace the cap on the control bottle.

(7) Repeat step (6) with the remaining control standards placing 100 µl amounts of these standards in the third, fourth, and fifth wells, respectively. A new pipette tip should be used for each standard solution.

(8) Firmly place a new pipette tip on the 100 µl pipette and add 100 µl from the sample collection tube of the first sample to the sixth well. Discard the tip.

(9) Repeat step (8) for each sample, placing 100 µl of extract from each sample in a different well. Use a new pipette tip for each sample solution.

(10) Using a 12-channel pipettor with new tips, mix the wells by pipetting the liquid up and down in the tips three times. Transfer 100 µl to the antibody wells.

(11) Mix by sliding the Microwell holder back and forth on flat surface in a manner to ensure mixing (10-20 seconds) without splashing reagents from wells. **Wait 10 minutes** (begin time after mixing). Discard the red-marked wells.

(12) The initial reaction is now completed. Shake out the contents of antibody-coated wells.

(13) Using a wash bottle, fill each antibody-coated well with distilled water and shake out. Repeat five times. Remove all water droplets by turning the wells upside down and vigorously tapping wells on a paper towel.

(14) Pipette 3 ml of substrate into the reagent boat, and with new tips on the 12-channel pipettor, pipette 100 µl of substrate into the wells and mix as instructed in step (11). **Wait 10 minutes** (begin time after mixing).

(15) Discard the remaining substrate and rinse the reagent boat with water.

(16) Pipette 3 ml of stop solution into the reagent boat. Using the same pipette tips as were used to dispense substrate, add 100 µl red stop to each well and mix thoroughly as instructed in step (11). Discard the tips.

b. Reading the Results.

**NOTE: Connect the Microwell reader to a computer system. For FGIS computers, the computer must have the necessary software installed on the C drive, subdirectory "DON." Perform the following (computer procedures may vary for official agencies depending on how the software is installed).**

(1) Allow the Microwell Model EL 301 Strip Reader to warm up for a minimum of 15 minutes before using.

(2) Turn on the computer.

(3) At the C:\> prompt, type in CD\DON and press the "Enter" key.

(4) At the C:\DON> prompt, type LL and press the "Enter" key.

This will bring the MAIN MENU of the Log/Logit program on the computer screen.

(5) Type A to select the "Run Log/Logit Program" option.

(6) At "Please Enter the Number of Standards:" type 5 and press the "Enter" key.

(7) At "Enter Standard Units:" type ppm and press the "Enter" key.

(8) At "Standard 2 Concentration:" type the concentration level (e.g., 0.5) and press the "Enter" key.

(9) At "Standard 3 Concentration:" type the concentration level (e.g., 1) and press the "Enter" key.

(10) At "Standard 4 Concentration:" type the concentration level (e.g., 2) and press the "Enter" key.

(11) At "Standard 5 Concentration:" type the concentration level (e.g., 6) and press the "Enter" key.

(12) If all values are correctly entered, press the F1 key. If they are not, press the E key and follow the instructions on the screen to edit values. When all values are correct, hit the F1 key.

**STOP! Do not use the computer keyboard until the samples have been read in step (21).**

(13) On the Microwell reader, remove the sample carriage and press the "Enter" key on the Microwell reader.

(14) Insert the W2 filter (405 nm) and press the "Enter" key.

(15) Insert the W1 filter (650 nm) and press the "Enter" key.

(16) Press "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(17) Load the antibody-coated wells into sample carriage so that the control labeled 0 (zero) is in position A1.

(18) Load the sample carriage into the strip reader so that position A1 is under the reader.

(19) Press "Read" and an absorbance value for A1 should appear in the screen on the Microwell reader.

(20) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear.

(21) Repeat step (20) until absorbance values have been obtained for all controls and samples.

(22) Return to the computer keyboard and type in "R." A message appears that tells you to “press data out on reader now!”

(23) Press the "Data Out" key on the Microwell reader. This will cause the collected data to be transferred to the computer.

(24) Enter a sample number for each sample and press the "Enter" key.

(25) When the last sample number is entered, hit the "Enter" key and the calculated ppm for each standard and sample will appear on the screen.

(26) Record the results for each sample along with the correlation coefficient, slope, and y-intercept data on a data sheet.

**NOTE: The correlation coefficient values must read .98 or higher to ensure accurate results. If the correlation value is less than .98, rerun the test. In addition, contact Neogen if the correlation coefficient is consistently below .98. Moreover, the slope value must read -2.0 (+ OR - 0.5).**

**If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings. Do not certify results if the correlation coefficient is less than 0.98 or the slope value is out of tolerance.**

c. Reporting and Certifying the Results

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 5.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/ deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

## 5.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 5.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

## 5.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits:

(1) monoclonal antibody-coated microwells.

(2) red-marked mixing wells.

(3) yellow-labeled bottle(s): DON control.

(4) blue-labeled bottles: conjugate solution.

(5) squeeze tubes: hydration solution.

(6) green labeled bottle: substrate solution.

(7) red-labeled bottle: stop solution.

b. Materials Required but not Provided:

(1) Mixing Bags -18-ounce Nasco Whirlpack bags; Fisher Scientific No. 01-812-6C, or similar type of sealable plastic bag.

(2) Nalgene funnels - 80 mm top I.D., stem 30 mm, stem O.D. 18 mm; American Scientific Products No. F7465-2.

(3) Plastic beakers - 250 ml plastic.

(4) Cylinders - Polypropylene, graduated, 250 ml capacity.

(5) Carboy - Nalgene, polyethylene, with spigot, 2 gallon capacity; Fisher Scientific No. 02-936-6A.

(6) Filter paper - 24 cm diameter; Whatman No. 1, or equivalent.

(7) Timer - 10-minute capacity.

(8) Markers - Sharpie or equal (permanent ink that will not wash off).

(9) Absorbent material - Kim wipes or paper towels.

(10) Wash Bottle 250 ml plastic squeeze bottle.

(11) Microwell Strip Reader Model EL301.

(12) IBM Compatible Computer.

(13) Multichannel Pipettor - TiterTek 12 channel or equivalent.

(14) Pipettor and Pipette Tips (100 µl) - Pipetteman, MLA or equivalent.

(15) Pipettor and Pipette Tips (1ml) - Pipetteman, MLA or equivalent.

(16) Microwell Holder.

(17) Deionized or distilled water.

(18) Strand Sizer or similar type shaking device.

(19) Whirlpack Bag Rack; Fisher Scientific No. 01-812-5E, or equivalent.

(20) Bond Elut SPE Cartridge, CN-E, 100 mg/1ml; Varian Sample Preparation Products No. 1210-2007 (For malted barley only).

(21) Vac Elut 10 with collector rack for 16 x 100 mm test tubes; Varian Sample Products No. 1223-4039 (For malted barley only).

(22) Balance.

(23) Sample Grinder.

## 5.10 STORAGE CONDITTIONS

Test kits should be refrigerated at temperatures between 36° F and 46° F.

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# CHAPTER 6

## NEOGEN - VERATOX 5/5 DON TEST KIT

## 6.1 TESTING AREA

The extraction solution and other materials used in the Veratox 5/5 test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

## 6.2 EXTRACTION PROCEDURES

a. Standard Extraction Procedure - Testing Wheat, Oats, Barley, Malted Barley and Corn Tested at the 5 ppm Conformance Limit

(1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.

(2) Label the collection container with the sample identification.

(3) Thoroughly mix the ground sample and weigh a 50-gram portion.

(4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.

(5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.

(6) Let the extract sit for 2 minutes to enable some of the sample to settle before filtering the extract.

(7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a cuvette.

(8) Dilute the sample extract 1:2 (1+1) with deionized or distilled water. (For example, add 1.0 ml of extract to 1.0 ml of deionized or distilled water).

(9) Mix well.

(10) Proceed to test analysis steps.

b. Optional Procedure - Testing Wheat, Oats, Barley, Malted Barley, and Corn at Lower Concentration Levels (between 0.5 - 2.5 ppm).

**NOTE: Using the optional extraction method limits the testing range from 0.5 ppm to 2.5 ppm. Any test result above 2.5 ppm is reported as > 2.5 ppm unless a supplemental analysis is performed.**

(1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.

(2) Label the collection container with the sample identification.

(3) Thoroughly mix the ground sample and weigh a 50-gram portion.

(4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.

(5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.

(6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.

(7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a cuvette.

(8) Proceed to the analysis steps.

## 6.3 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one hour).

(2) Remove one red-marked mixing well for each sample to be tested, plus five red-marked wells to be used for controls. Place these wells in the microwell holder.

**NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the controls and the remainder of the wells for test samples.**

(3) Remove an equal number of antibody-coated wells. Immediately return antibody wells that will not be used to the foil pack with desiccant. Fold down ends of the pack and seal with tape to protect the antibody. Mark one end of the strip so that the wells can be identified after washing.

(4) Mix each reagent by swirling the reagent bottle prior to use.

(5) Using a new pipette tip for each, transfer 100 µl of conjugate from the blue-labeled bottle into each mixing well.

(6) Using a new pipette tip for each, transfer 100 µl of control and sample extract into the mixing wells as shown below:

| Well # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | C 0 | C .25 | C .5 | C 1.0 | C 3.0 | S1 | S2 | S3 | S4 | S5 | S6 | S7 |

Where C 0 is the zero control, C .25 is the .25 ppm control, C .5 is the 0.5 ppm control, C 1.0 is the 1.0 ppm control, and C 3.0 is the 3.0 ppm control. S1 is sample 1, S2 is sample 2, etc.

(7) Using a 12- channel pipettor, mix the wells by pipetting the liquid up and down in the tips 3-4 times. Transfer 100 µl to the antibody wells and mix by sliding the microwell holder back and forth on a flat surface for 10 –20 seconds without splashing reagents from the wells. Incubate for **5 minutes** at room temperature (64 –86º F). Discard the red-mixing wells.

(8) Dump the contents of the antibody wells. With a wash bottle or a running stream, fill each antibody well with deionized or distilled water and then dump the water out. Repeat this step 5 times, then turn the wells upside down and tap out on a paper towel until the remaining water has been removed.

(9) Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat, and with new tips on the 12-channel pipettor, prime and pipette 100 µl of substrate into the wells and mix by sliding back and forth on a flat surface for 10-20 seconds. Incubate for **5 minutes.** Discard the remaining substrate and rinse the reagent boat with water.

(10) Pour the Red Stop solution from the red-labeled bottle (same volume as prepared for substrate) into the red-label reagent boat. Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 µl of the Red Stop to each well. Mix by sliding back and forth on a flat surface. Discard the tips.

(11) Wipe bottom of microwells with a dry cloth or towel and read in the Awareness Stat-Fax Model 321 PLUS Reader using a 650 nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes of completion of the test.

b. <u>Reading the Results with the Stat-Fax Model 321 PLUS Microwell Reader.</u>

To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter. For DON, the Veratox test number is 5.

(1) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(2) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(3) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

(4) If a diluted sample extract (see Standard Extraction Procedure) is being analyzed, the reader value for the extract will need to be modified to adjust for the dilution of the extract. If the original extract was diluted 1+1 with water (this is an actual 1:2 dilution), the sample results are multiplied by 2. If the original extract was diluted 1+3 with water (this is an actual 1:4 dilution), the sample results are multiplied by 4.

(5) If the Optional Extraction Procedure was used for testing samples at lower concentration levels (0.5 - 2.5 ppm) samples, the reader values do not need to be adjusted for dilution of the extract because an undiluted extract was analyzed.

**NOTE: If the correlation coefficient is less than 0.98 or if the slope exceeds –2.0 (± 0.5), the reader will print, "Invalid Calibration" and no results will be reported. If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings.**

## 6.4 REPORTING AND CERTIFYING TEST RESULTS

a. Testing Wheat, Oats, Barley, Malted Barley and Corn Tested at the 5 ppm Conformance Limit using the Standard Extraction Procedure

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

b. Testing Wheat, Oats, Barley, Malted Barley and Corn Tested at the 2.5 ppm Conformance Limit using the Optional Extraction Procedure

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 2.5 ppm. Results exceeding 2.5 ppm are reported as > 2.5 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 2.4 ppm are certified to the nearest whole ppm.

Test results that are equal to the conformance limit (2.5 ppm) are certified as being equal to 2.5 ppm.

Test results over 2.5 ppm are certified as exceeding 2.5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 6.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** (5 ppm for the normal procedure and 2.5 ppm for the optional procedure) is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON value at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

## 6.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 6.7 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

## 6.8 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits:

(1) 48 Monoclonal-Coated microwells.

(2) 48 Red-Marked Mixing Wells.

(3) 5 Yellow labeled Bottles - 1.5 ml each of 0, 0.25, 0.5, 1.0, and 3.0 ppm DON Controls.

(4) 1 Blue-Labeled Bottle - DON-HRP Conjugate Solution.

(5) 1 Green-Labeled Bottle - 24 ml K-Blue Substrate Solution.

(6) 1 Red-Labeled Bottle - 32 ml Red Stop Solution.

b. Materials Required but not Provided:

(1) Extraction Materials: Whirlpack Bags -18 oz., or equivalent.

(2) Microwell Strip Reader: Awareness Technology STAT-FAX Model 321 PLUS Reader with a 650 nm filter.

(3) 12-Channel multi-channel pipettor and pipette tips.

(4) 100 µl pipettor and pipette tips.

(5) 100 ml pipettor and pipette tips.

(6) Deionized or Distilled Water.

(7) Absorbent Materials: Paper Towels, Kay Dry paper or equivalent.

(8) Waste receptacle.

(9) Microwell holder.

(10) Timer: 3-channel minimum.

(11) Waterproof marker: Sharpie or equivalent.

(12) Wash Bottle.

(13) Reagent Boats.

(14) Sample grinder.

(15) Balance.

(16) Neogen Filter Syringe.

## 6.9 STORAGE CONDITIONS

Test kits should be refrigerated at temperatures between 36º F and 46º F.

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# CHAPTER 7

## ROMER - DON FLUOROQUANT TEST KIT

## 7.1 TESTING AREA

The extraction solution and other materials used in the Fluoroquant test kit necessitates the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

## 7.2 EXTRACTION PROCEDURES

a. Preparation of Extraction Solvent (84 Percent Acetonitrile Solution).

Make up the solution by using the ratio of 84 parts acetonitrile to 16 parts deionized/distilled water. Prepare the 84 percent acetonitrile solution by adding 840 ml acetonitrile to 160 ml of distilled or deionized water. Mix well. Label the solution bottle and keep it tightly capped when not in use.

If the amount of solution being prepared needs to be adjusted based on the workload at individual locations, make sure that 84 parts acetonitrile to 16 parts deionized/distilled water ratio is maintained.

b. Extraction Procedures.

(1) Place 50 grams of ground sample into a blender jar.

(2) Add 200 ml of acetonitrile/water (84/16) and blend on high for 3 minutes.

(3) Filter into a sample container using coffee filters or Whatman No. 1 filter paper.

## 7.3 TEST PROCEDURES

a. Purification Procedures.

**NOTE: All solution transfers may be carried out using adjustable automatic pipettors with disposable tips. Care should be taken to make sure that the tips used are large enough to hold the volume being transferred. Make sure that they are securely attached to the pipettor.**

(1) Place 4 ml of extract in a 15 x 85 culture tube. Insert a MycoSep #225 column into the top of the culture tube and slowly (20 seconds) push to the bottom of the tube.

**(Note: Use 6 ml of extract and a MycoSep #227 column for malted barley samples and take 30 seconds to push the extract through the column.)**

(2) Transfer 1.5 ml of each purified sample extract to a 12 x 75 mm cuvette. Use a clean pipette tip for each transfer.

b. Calibrators and Control Preparation.

(1) Allow the calibrator and control solutions to come to room temperature.

(2) Invert each calibrator standard bottle and control standard bottle three times to mix thoroughly.

(3) Transfer 1.5 ml of the green-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(4) Using a clean tip, transfer 1.5 ml of the red-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(5) Using a clean tip, transfer 1.5 ml of the control (yellow-labeled) solution to a clean 12 x 75 mm cuvette.

(6) Cap the calibrator solutions tightly and store in the refrigerator.

(7) Proceed with the analysis, treating samples, calibrators, and control identically.

c. Evaporation Procedures.

Evaporate each sample, calibrator, and control to dryness using a vacuum manifold and dry bath set at 70°C.

**Note: To decrease the evaporation time, turn off the vacuum to the manifold for the rows that are not being used.**

d. Derivatization Procedures.

(1) Add 1.5 ml of Reagent A to all sample tubes, calibrators, and control.

(2) Add 50 microliters (µl) of Reagent B to all sample tubes, calibrators, and control. Cap the tubes and mix contents with a vortex for 10 seconds.

(3) Heat the tubes in a 50°C bath for 10 minutes.

(4) Remove tubes from the bath and cool to room temperature. Read the samples in the fluorometer within 1 hour.

**Note: Cuvettes may be placed in tap water for 30 seconds to cool. Dry the outside wall of the cuvette completely before placing in the fluorometer.**

e. Fluorometer Reading.

Calibrate the fluorometer using the following procedure:

(1) Turn on the power (no warm-up is necessary).

(2) Change the date or time - If correct, press the "Continue" key.

(3) When asked for Test Delay Time, enter "2" and press the "Enter" key.

(4) When asked for answer format, select "Decimals."

(5) When asked for measurement units, select "ppm."

(6) At the "insert red vial" prompt, place the appropriate calibrator cuvette into the sample well.

(7) When asked for the calibrator value, enter the appropriate value (refer to the card supplied with the calibrators for the red value) and press the "Enter" key.

(8) When asked to "remove the red vial," remove the calibrator tube from the sample well.

(9) At the "insert green vial" prompt, place the appropriate calibrator cuvette into the sample well.

(10) When asked for the "blank value, "enter the appropriate value (refer to the card supplied with the calibrators for the green value) and press the "Enter" key.

(11) When asked to "remove the green vial," remove the calibrator tube from the sample well.

(12) At the "insert test vial" prompt, place the control cuvette into the sample well.

(13) The fluorometer will now display the value for the control vial.

(14) Compare the value of the control with the values listed on the card. If the control value is within the specified range, the fluorometer is ready to analyze samples. If the value is outside of the specified range, rerun the red, green, and yellow calibration cuvettes. If the control value still exceeds the specified range limit, contact Romer Labs.

(15) Press the "Enter" key. The fluorometer is now ready to analyze samples.

f. Reading the Results.

To determine the DON concentration insert the cuvette containing the sample portion into the sample well of the fluorometer. The DON concentration will appear on the display after the appropriate 2-second delay. Read the results.

## 7.4 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 7.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (acetonitrile/water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

## 7.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 7.7 WASTE DISPOSAL

Transfer sample extract solutions (acetonitrile/water) and derivatization solutions into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Transfer sample slurry, used filter paper, cuvettes, caps, and columns into the normal solid waste container for routine disposal.

## 7.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) glass culture tubes (15 x 85 mm) - 25 tubes per test kit

(2) MycoSep #225 (or #227 for malted barley) Columns

(3) glass cuvettes and caps (12 x 75 mm) - 50 per test kit

(4) Reagent A - Ethylenediamine in methanol

(5) Reagent B - Zirconyl Nitrate in methanol

(6) Calibrator solutions (red-label, and green-label) plus control solution (yellow-label)

b. Materials Required but not Provided:

(1) Blender - Oster Mixer, Model 848-31A or Waring Blender with S.S. blender container or similar, the unit must be explosion proof.

(2) Cutting Assembly - Process unit with sealing ring for Oster Mixer, Model 848-31A; Oster Corp. 937-45 or Eberbach blender jar or similar.

(3) Bottom Cap - Threaded for Oster Mixer, Model 848-31A; Oster Corp. No. 937-46.

(4) Square type jar - Designed to fit above.

(5) Nalgene funnels - 80 mm top I.D., stem 30 mm, stem O.D. 18 mm; American Scientific Products No. F7465-2.

(6) Plastic beakers - 250 ml plastic.

(7) Cylinders - Polypropylene, graduated, 250 ml capacity.

(8) Carboy - Nalgene, polyethylene, with spigot, 2 gallon capacity; Fisher Scientific No. 02-936-6A

(9) Extraction Solvent - Acetonitrile/distilled or deionized water (84/16).

(10) Filter Paper - standard coffee filters or Whatman No. 1

(11) Fluorometer with printer - Romer RL 100 (# FQ 1002) or equivalent (Vicam Series III and IV, Torbex 100, Vicam FX-100).

(12) Vortex Mixer - Romer #EQP 8113.

(13) Vacuum Pump and Trap - Romer # EQP 8106.

(14) E-Vap Evaporator - Romer # EQP8188.

(15) Test Tube Rack - Romer # EQP 8193.

(16) Thermometer - Romer # EQP 8174.

(17) Dry Bath with Heating Block - Romer # EQP 8180 / EQP 8181.

(18) Pipette and tips - 50 µl.

(19) Pipette and tips - 1.5 ml.

(20) Sample grinder.

(21) Balance.

## 7.9 STORAGE CONDITIONS

a. Columns.

MycoSep #225 and #227 columns - Room temperature in a drawer or box.

b. Reagents.

Reagents A & B are shipped in amber bottles, cap tightly and store in a temperature controlled area (between 40° and 80° F). Do not freeze. Reagents should stay stable for 6 months.

c. Calibration and Control Solutions.

Calibration and control solutions are shipped in amber vials, cap tightly and store in the refrigerator. Solutions should stay stable for 6 months.

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# CHAPTER 8

## ROMER - ACCUTOX™ DON TEST KIT

## 8.1 TESTING AREA

The extraction solution and other materials used in the AccuTox™ test kits does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

## 8.2 EXTRACTION PROCEDURES

a. Place 50 grams of ground sample into a clean plastic or glass container.

b. Add 250 milliliters (ml) of distilled or deionized water.

c. Seal or cover the mixing container and shake (by hand or mechanically) for 3 minutes.

d. Allow the sample residue to settle.

e. Unseal or remove the cover from the container and pour the extract through filter paper (standard coffee filters or Whatman No.1) into a sample jar labeled with the sample identification.

## 8.3 TEST PROCEDURES

a. Sample Analysis.

(1) Allow reagents, antibody coated tubes, and sample extracts to reach room temperature prior to running test (approximately 1 hour).

(2) Place the appropriate number of labeled antibody-coated tubes into the gripper tube rack. Reseal the unused tubes in a zip-lock bag with desiccant.

(3) Pipette 0.5 ml of the zero calibrator, control, and samples directly into the bottom of the antibody coated tubes without touching the sidewalls. Completely discharge the pipette by depressing the plunger with the thumb to the second stop (all the way down).

(4) Pipette 0.5 ml of the enzyme conjugate into each tube. The conjugate must be pipetted (completely releasing the thumb after each addition) down the sides of the tubes. Start a timer set for 15 minutes as soon as conjugate has been added to the first tube.

(5) Shake the rack using a circular motion for approximately 5 seconds.

(6) At the completion of the 15-minute incubation period, dump the contents of the tubes into the appropriate waste container. Fill the tubes to overflowing and forcibly rinse with the wash solution. Completely empty the tubes after the rinse. Repeat this process three more times for a total of four washes. (It is very important not to underwash the tubes. Over washing will not affect the test.)

(7) Following the last wash, invert the tubes and forcibly tap onto absorbent paper several times to remove all of the wash solution. (It is important to remove as much wash as possible.)

(8) Pipette 0.5 ml of the substrate into each tube. The substrate must be pipetted (completely releasing the thumb after each addition) down the sides of the tubes. Start a timer set for 5 minutes as soon as the substrate has been added to the first tube. Swirl the rack in a circular motion for approximately 5 seconds to mix. Solutions should all turn blue after substrate has been added.

(9) Add 0.5 ml of stop solution to each tube by pipetting down the sides of the tubes. Swirl the rack in a circular motion for approximately 5 seconds to mix. All solutions should turn yellow after adding the stop solution.

(10) Make sure that the spectrophotometer is set at 450 nm. Run a blank with a clean unscratched test tube filled with fresh distilled or deionized water.

(11) Zero the spectrophotometer prior to reading the tubes. Make sure that there are no air bubbles in the blank tubes before zeroing.

(12) Wipe each tube with a lint free towel before reading and allow a few seconds for the specrophotometer reading to stabilize before printing the absorbance level reading.

(13) Read and record absorbance levels of the calibrator, control, and samples.

(14) Calculate results using log/logit data computer program with factory calibration included with kit.

b. Spectrophotometer Calibration Procedure.

(1) Turn on the spectrophotometer by using the "ON/OFF" key. After turning on the power, the spectrophotometer goes through a self-test.

**Note: The lid must be closed.**

(2) Using the dial on the right side of the spectrophotometer, dial in 450 nm.

(3) The spec will then display "Enter Program #." At this time press 0 and the "Enter" button.

(4) P O will then be displayed. Take the blank tube filled half way with distilled or deionized water and place into the well. Cover the tube with the small cylinder cover supplied.

(5) Press the key labeled " Zero."

(6) The spectrophotometer is now ready for calibrators and samples.

(7) After placing calibrator or sample into the well and covering, wait a few seconds (1-5 seconds) for the reading to stabilize.

(8) Turn on the printer and verify that the printer on line light is lit. Press the "Shift" key, then the "Print" key (blue writing) to print the absorbance level readings.

## 8.4 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 8.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

## 8.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 8.7 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

## 8.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) Antibody coated tubes.

(2) Conjugate.

(3) Substrate.

(4) Zero calibrator standard.

(5) Control solution.

(6) Stop solution.

(7) Wash solution.

b. Materials Required but not Provided:

(1) Hach Spectrophotometer.

(2) 500 µl pipettor with tips.

(3) Gripper test tube rack.

(4) Timer - 15 minute capacity.

(5) Plastic squirt bottle for wash solution.

(6) Sealable plastic bags or plastic/glass containers with tight fitting lids.

(7) Distilled or deionized water.

(8) 100 ml graduated cylinder.

(9) Filter paper (standard coffee filters or Whatman No.1).

(10) Sample grinder.

(11) Balance.

## 8.9 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

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# CHAPTER 9

## DIAGNOSTIX - EZ-QUANT DON PLATE TEST KITS

## 9.1 GENERAL INFORMATION

FGIS has approved two separate Diagnositx test kits for quantitative DON testing. The EZ-Quant DON Plate test kit (part number 600312) is used for testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm. The EZ-Quant 0.5 PPM DON Plate test kit (part number 600313) is used for testing barley and malted barley from 0.5 to 2.5 ppm.

## 9.2 TESTING AREA

The extraction solution and other materials used in the Diagnostix EZ-Quant DON Plate test kits (part numbers 600312 and 600313) do not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in the handbook to ensure a safe and efficient work environment.

## 9.3 EXTRACTION PROCEDURES

The extraction procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

a. Place 50 grams of ground sample into a clean plastic bag.

b. Add 250 ml of distilled or deionzed water and seal/close the bag securely to prevent spillage.

c. Shake vigorously (by hand or mechanically) for three minutes.

d. Let the extract sit for 2-3 minutes to allow for some settling of the slurry.

e. Filter a minimum of 15 ml of the extract through Whatman #4 filters (or equivalent) into a clean container that is labeled with sample ID number.

## 9.4 PREPARATION OF SOLUTIONS

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

a. To prepare the Wash Solution, transfer the contents of the Wash Concentrate vial to a 500-ml container and add 475 ml of distilled or deionized water.

b. Swirl to mix.

## 9.5 TEST PROCEDURES

a. EZ-Quant DON Plate Test Kit (Part Number 600312) - Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

(1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one-hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder. Do not run more than two strips at a time. Be sure to re-seal unused wells in the zip-lock bag with desiccant.

**NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.**

(3) Dispense 100 µl of Enzyme Conjugate into each red mixing well.

(4) Dispense 100 µl of the appropriate calibrators and samples into the appropriate red mixing wells as illustrated below.

**NOTE: Use a clean pipette for each addition.**

| **Wells** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **First Strip** | **0** | **0.5** | **1.0** | **2.0** | **6.0** | **S** | **S** | **S** | **S** | **S** | **S** | **S** |
| **Second Strip** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** |

**Key : S= Sample, 0= 0 ppm calibrator, 0.5= 0.5 ppm calibrator, etc.**

(5) Using a 12-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips 5 times in the mixing wells.

(6) Using a 12-channel pipette, transfer 100 µl of the reaction mixture into the corresponding clear test wells and tap the holder several times to mix. Discard the red mixing wells into an appropriate waste container.

(7) Incubate the clear test wells for 10 minutes.

(8) Dump the contents of the wells into an appropriate waste container and carefully shake out any residue solution.

(9) Using a wash bottle filled with wash solution, fill each well to overflowing then dump the contents and shake out any residue solution. Repeat four times for a total of 5 washes.

(10) After the final wash, tap the strips repeatedly onto absorbent paper to remove excess wash. After tapping, check for large bubbles, which should be burst with a clean pipette tip and tapped out again.

(11) Using a 12-channel pipette, dispense 100 µl of Substrate into each well and tap the holder several times to mix the contents.

(12) Using a paper towel to block out the light reflectance, cover the wells and incubate for 5 minutes.

(13) Using a 12-channel pipette, dispense 100 µl of Stop Solution into each well.

(14) Within 10 minutes, read and record the absorbance of each well at 450 nm using the Bio-Tek EL 301™ Microwell Strip Reader equipped with a 450 nm filter.

b. <u>EZ-Quant 0.5 PPM DON Plate Test Kit (Part Number 600313) - Testing Barley and Malted Barley from 0.5 to 2.5 ppm</u>.

(1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one-hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder. Do not run more than two strips at a time. Be sure to re-seal unused wells in the zip-lock bag with desiccant.

**NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.**

(3) Dispense 100 µl of Enzyme Conjugate into each red mixing well.

(4) Dispense 100 µl of the appropriate calibrators and samples into the appropriate red mixing wells as illustrated below.

**NOTE: Use a clean pipette for each addition.**

| **Wells** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **First Strip** | **0** | **0.2** | **0.5** | **1.0** | **2.5** | **S** | **S** | **S** | **S** | **S** | **S** | **S** |
| **Second Strip** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** |

**Key : S= Sample, 0= 0 ppm calibrator, 0.5= 0.5 ppm calibrator, etc.**

(5) Using a 12-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips 5 times in the mixing wells.

(6) Using a 12-channel pipette, transfer 100 µl of the reaction mixture into the corresponding clear test wells and tap the holder several times to mix. Discard the red mixing wells into an appropriate waste container.

(7) Incubate the clear test wells for 10 minutes.

(8) Dump the contents of the wells into an appropriate waste container and carefully shake out any residue solution.

(9) Using a wash bottle filled with wash solution, fill each well to overflowing then dump the contents and shake out any residue solution. Repeat four times for a total of 5 washes.

(10) After the final wash, tap the strips repeatedly onto absorbent paper to remove excess wash. After tapping, check for large bubbles, which should be burst with a clean pipette tip and tapped out again.

(11) Using a 12-channel pipette, dispense 100 µl of Substrate into each well and tap the holder several times to mix the contents.

(12) Using a paper towel to block out the light reflectance, cover the wells and incubate for 5 minutes.

(13) Using a 12-channel pipette, dispense 100 µl of Stop Solution into each well.

(14) Within 10 minutes, read and record the absorbance of each well at 450 nm using the Bio-Tek EL 301™ Microwell Strip Reader equipped with a 450 nm filter.

c. Reading the Results.

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

(1) Allow the EL 301 Microwell reader to warm up for a minimum of 15 minutes before using.

(2) Remove the sample carriage and hit "Enter."

(3) Insert W2 filter (405 nm) and hit "Enter."

(4) Insert W1 filter (450nm) and hit "Enter."

(5) Hit "Clear"and "Blank." This will cause the instrument to read air as the blank sample.

(6) Load the anti-body coated wells into the carriage so that the control labeled 0 (zero) is in position A1.

(7) Load the sample carriage into the well reader so that position A1 is under the reader.

(8) Hit "Read" and record the absorbance value obtained for A1 in the screen of the Microwell reader.

(9) Slide the carriage to position A2 and hit "Read." Record the absorbance value obtained for A2.

(10) Repeat step (9) until absorbance values have been obtained for all controls and samples.

(11) Use the Data Reduction software provided by Diagnostix to convert the absorbance values into concentration values.

d. Interpretation of Results - (Diagnostix Data Reduction Worksheet Program)

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

To generate a calibration curve and convert sample absorbances into DON concentrations, use the Diagnostix Data Reduction Worksheet program provided by Diagnostix. A computer equipped with the Microsoft Excel program is required to run the EZ-Quant DON Plate Kit Data Reduction Worksheet.

(1) Place the diskette provided into the appropriate drive. Open the Microsoft Excel program, access the diskette, and click on **"Data Reduction Worksheet."**

(2) Complete the following information on the worksheet:

(a) Operator (b) Date

(c) Assay ID (d) Kit Lot #

(3) Under the **"Absorbance"** column in **"Section 1 Calibrator Curve"**, input the Standard absorbance values to the appropriate designated rows.

After the absorbance values are added, the worksheet automatically calculates $B/B_o$, then plots a calibration curve in the graph field (Log (standard concentration) on the abscissa, Logit $B/B_o$ on the ordinate). In addition $R^2$, Slope and Intercept values are calculated.

(4) Under the **"Abs."** column in **"Section II - Sample Calculations"**, input the absorbance value for each sample tested (the worksheet provides enough space for 19 samples). A **"Sample ID"** column is provided to the left of the "Abs." column to identify each sample.

After the sample absorbance values are added, the worksheet automatically calculates the concentration in parts per million (ppm) and presents them in the "ppm" column.

(5) Repeat steps (2), (3), and (4) for each new set of data.

e. Validation Requirements - EZ-Quant DON Plate Test Kit (Part Number 600312) Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

For a given test to be considered valid, the 0 ppm standard should have an absorbance greater than 0.600 and the calibration curve must meet the following two criteria:

(1) The $R^2$ value should be between -0.992 and -1.000.

(2) The $B/B_o$ values for the standards should fall within the following range.

| Standard | Acceptable Range |
|---|---|
| 0.5 ppm | 0.67 - 0.80 |
| 1.0 ppm | 0.53 - 0.65 |
| 2.00 ppm | 0.38 - 0.49 |
| 6.00 ppm | 0.18 - 0.28 |

**NOTE: If the $R^2$ value on the $B/B_o$ values for the standards fall outside the specified range call Diagnostix at 1-800-282-4075 for assistance.**

f. Validation Requirements - EZ-Quant DON Plate Test Kit (Part Number 600313) Testing Barley and Malted Barley from 0.5 to 2.5 ppm

For a given test to be considered valid, the 0 ppm standard should have an absorbance value between 0.6 and 2.5 and the calibration curve must meet the following two criteria:

(1) The $R^2$ value should be between -0.992 and -1.000.

(2) The $B/B_o$ values for the standards should fall within the following range

| Standard | Acceptable Range |
|---|---|
| 0.2 ppm | 0.70 - 0.74 |
| 0.5 ppm | 0.47 - 0.55 |
| 1.0 ppm | 0.35 - 0.40 |
| 2.5 ppm | 0.17 - 0.26 |

**NOTE: If the $R^2$ value on the $B/B_o$ values for the standards fall outside the specified range call Diagnostix at 1-800-282-4075 for assistance.**

## 9.6 REPORTING AND CERTIFYING TEST RESULTS

a. EZ-Quant DON Plate Test Kit (Part Number 600312) - Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

b. EZ-Quant DON Plate Test Kit (Part Number 600313) - Testing Barley and Malted Barley from 0.5 to 2.5 ppm

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 2.5 ppm. Results exceeding 2.5 ppm are reported as > 2.5 ppm

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 2.4 ppm are certified to the nearest whole ppm.

Test results that are equal to the conformance limit (2.5 ppm) are certified as being equal to 2.5ppm.

Test results over 2.5 ppm are certified as exceeding 2.5 ppm.

Refer to the Certification section of the handbook for more detailed certification procedures.

**NOTE: A supplemental analysis (diluting the mixture) is not allowed to obtain results above the 2.5 ppm conformance limit. To obtain accurate results above the 2.5 ppm conformance limit the sample must be tested using a test kit with a conformance range between 0.5 - 5 ppm.**

## 9.7 SUPPLEMENTAL ANALYSIS

A supplemental analysis is applicable only to the EZ-Quant DON Plate Test Kit (Part Number 600312) - Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/ deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

## 9.8 CLEANING LABWARE

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 9.9 WASTE DISPOSAL

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain followed by copious amounts of water. Discard solid material in the trash can for routine disposal.

## 9.10 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits: EZ-Quant DON test kit (part number 600312)

(1) 96 antibody-coated clear microtiter wells and 96 red mixing microtiter wells.

(2) 5 vials, each containing 4 ml of DON calibrators corresponding to 0, 0.5, 1.0, 2.0, and 6 µg/ml (ppm) of DON. (Note: because of the 1:5 dilution of the grain sample in the extraction step, the calibrator actually contains 1/5 of the stated value. No further correction back to the concentration in the original grain sample is required).

(3) 1 Vial containing 12 ml of DON-HRP Enzyme Conjugate.

(4) 1 Vial containing 12 ml of Substrate.

(5) 1 Vial containing 12 ml of Stop Solution. (Caution! 1 N HCL Handle with care).

(6) 1 Vial containing 25 ml of 20X Wash Solution.

(7) Strip holder (provided separately by Diagnostix).

(8) Data reduction program (provided separately by Diagnostix).

b. Materials Supplied in Test Kits: EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

(1) 96 antibody-coated clear microtiter wells and 96 red mixing microtiter wells.

(2) 5 vials, each containing 4 ml of DON calibrators corresponding to 0, 0.2, 0.5, 1.0, and 2.5 µg/ml (ppm) of DON. (Note: because of the 1:5 dilution of the grain sample in the extraction step, the calibrator actually contains 1/5 of the stated value. No further correction back to the concentration in the original grain sample is required).

(3) 1 Vial containing 12 ml of DON-HRP Enzyme Conjugate.

(4) 1 Vial containing 12 ml of Substrate.

(5) 1 Vial containing 12 ml of Stop Solution. (Caution! 1 N HCL Handle with care).

(6) 1 Vial containing 25 ml of 20X Wash Solution.

(7) Strip holder (provided separately by Diagnostix).

(8) Data reduction program (provided separately by Diagnostix).

c. Materials Required but not provided in the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

(1) Laboratory quality distilled or deionized water.

(2) Graduated cylinder, 1000 ml.

(3) Sealable containers for sample extraction and extract collection.

(4) Filter paper, Whatman No. 4 or equivalent.

(5) Pipette capable of dispensing 100 µl.

(6) 12-Channel pipette capable of dispensing 100 µl per channel.

(7) Disposable tips compatible with pipettes.

(8) Paper towels or equivalent absorbent materials.

(9) Bio-Tek EL 301™ Microwell Strip Reader with 450 nm filter.

(10) Timer.

(11) Reagent reservoirs.

(12) Wash bottle.

(13) Balance.

(14) Sample Grinder.

## 9.11 STORAGE CONDITIONS

The storage conditions listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

On the day the kit will be used it is acceptable to store the kit at ambient temperature (62° F to 82° F).

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# CHAPTER 10

## r-BIOPHARM RIDASCREEN®FAST DON TEST KIT

## 10.1 TESTING AREA

The extraction solution and other materials used in the r-Biopharm RIDASCREEN®FAST DON test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in the handbook to ensure a safe and efficient work environment.

## 10.2 EXTRACTION PROCEDURES

a. Place 50 grams of ground sample into a suitable container (e.g., plastic bag).

b. Add 250 ml of distilled/deionized water and seal/close container securely to prevent spillage.

c. Shake vigorously (by hand or mechanically) for three minutes.

d. Let the extract sit for 2-3 minutes to allow for some settling of the slurry.

e. Filter the extract through Whatman #1 filters (or equivalent) into a clean container that is labeled with sample ID number.

f. Dilute the filtered extract with one part sample extract to 3 parts distilled/deionized water. (e.g., 1 ml sample extract plus 3 ml water)

g. Use 50 µl of the diluted filtrate per well in the test.

## 10.3 PREPARATION OF SOLUTIONS

a. To prepare the Wash Solution, dissolve the contents of the packet containing the buffer salt in 1 liter of distilled water.

b. Swirl to mix.

When stored properly (at 39º F) the solution has a shelf life of four weeks.

## 10.4 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents and antibody wells to reach room temperature (68 - 77º F) prior to running the test.

(2) Insert a sufficient number and wells into the microwell holder for all standards and samples to be tested. (For example: to test 11 samples use 16 wells - 5 for the standards and 11 for the test samples).

Test Strip #1

| Well # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Sample | C 0 | C .222 | C .666 | C 2.0 | C 6.0 | S1 | S2 | S3 |

Test Strip #2

| Well # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Sample | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 |

Where C 0 is the zero control, C .222 is the 0.222 ppm control, C .666 is the 0.666 ppm control, C 2.0 is the 2.0 ppm control, and C6 is the 6.0 ppm control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

**NOTE: Do not run more than 3 strips (19 samples) per set of control standards.**

(3) Using a new pipette tip for each standard and sample, pipet 50 µl of standards and prepared sample to separate wells.

(4) Add 50 µl of enzyme conjugate (red capped bottle) into each well.

(5) Add 50 µl of deoxynivalenol antibody (black capped bottle) into each well.

(6) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(7) Incubate for 5 minutes (± 1 minute) at room temperature.

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(9) Using a wash bottle, fill each well with washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(10) Add 100 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(12) Incubate for 3 minutes (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(15) Measure absorbance at 450 nm using the Biotek EL 301, Awareness Technology Stat-Fax Model 303 PLUS, or the Hyperion Microreader™ 3 Model 4027-002, microwell readers.

(Results must be read within 10 minutes)

b. Reading the Results.

(1) Biotek EL 301 Microwell Reader.

(a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.

(b) Remove sample carriage and hit "Enter."

(c) Insert W2 filter and hit "Enter."

(d) Insert W1 filter (450 nm) and hit "Enter."

(e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(f) Load antibody-coated wells into sample carriage so that the first control labeled 0 is in position A1.

(g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.

(h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.

(i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.

(j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.

(k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

(2) Stat-Fax Model 303 PLUS Microwell Reader

(a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter.

(b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

(d) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

(3) Hyperion Microreader™ 3 Model 4027-002 Microwell Reader.

(a) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.

(b) When prompted to "Run a test", select yes, select the appropriate test number, then press "Enter".

(c) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24) then press "Enter".

(d) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.

(e) The reader will read the optical density of the wells and print a report.

(f) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the to the instrument prompts as indicated above.

(g) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

## 10.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 10.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON value at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original diluted filtrate (obtained from step f., section 10.2) with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

## 10.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 10.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

## 10.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (48 well kit).

(1) 1 microtiter plate.

(2) 48 Antibody coated wells.

(3) 5 DON standard solutions of 1.3 ml each; 0, 0.222, 0.666, 2.0, and 6.0 ppm DON in water.

(4) 1 red-capped bottle of 3 ml peroxidase conjugated deoxynivalenol solution.

(5) 1 black-capped bottle of 3 ml anti- deoxynivalenol antibody.

(6) 1 white dropper bottle of 6 ml Substrate/Chromagen, stained red.

(7) 1 yellow or orange dropper bottle of Stop reagent.

(8) 1 packet of washing buffer (salt).

b. Materials Required but not Provided.

(1) Biotek EL 301 Microwell Reader, Awareness Technology Inc. Stat-Fax Model 303 PLUS, or Hyperion MicroReader™ 3 Model No. 4027-002 with 450-nm filters.

(2) RIDA™SOFT Win Software.

(3) 50 µl, 100 µl, and 1000 µl Pipettor and pipette tips.

(4) Graduated cylinders (plastic or glass): 100 ml, 1 liter.

(5) Sample shaker (optional).

(6) Filter funnel.

(7) Whatman #1 filter paper or equivalent.

(8) Balance.

(9) Stepper pipetter.

(10) Paper towels, Kaydry paper or equivalent absorbent material.

(11) Waste receptacle.

(12) Timer: 3 channel minimum.

(13) Waterproof marker, Sharpie or equivalent.

(14) Wash bottle.

(15) Deionized or distilled water.

## 10.10 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

When stored properly (at 39º F) the Wash Solution has a shelf life of four weeks.

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# CHAPTER 11

## STRATEGIC DIAGNOSTICS INC. - MYCO✔ DON TEST KIT

## 11.1 TESTING AREA

The extraction solution and other materials used in the Myco✓ DON test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

## 11.2 EXTRACTION PROCEDURES

a. Weigh 50 grams of ground sample and place in a clean blender container with a tight fitting lid.

b. Add 250 ml of distilled or deionized water.

c. Blend at high speed for 2 minutes.

d. Allow the extract to stand for 2-3 minutes to allow the sample slurry to settle.

e. Filter a minimum of 15 ml of the extract through a Whatman #1 filter and collect the extract in a clean container that is labeled with the sample ID number.

f. Proceed to the test procedures.

## 11.3 PREPARATION OF SOLUTIONS

a. To prepare the Wash Solution, transfer the contents of the Wash Concentrate vial to a 500 ml plastic squeeze bottle and add 475 ml of distilled or deionized water.

b. Swirl to mix.

## 11.4 TEST PROCEDURES

a. Analysis Procedure.

(1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder.

(3) Dispense 100 µl of enzyme conjugate into each well using a pipette.

(4) Using a clean pipette tip for each transfer, dispense 100 µl of each calibrator and sample into the appropriate mixing wells using an adjustable or fixed 100 µl pipette.

| Well # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | C 0 | C .25 | C .5 | C 1 | C 3 | S1 | S2 | S3 | S4 | S5 | S6 | S7 |

Where C 0 is the zero control, C .25 is the 0.25 ppm control, C .5 is the 0.5 ppm control, C 1 is the 1.0 ppm control, and C3 is the 3.0 ppm control. S1 is sample 1, S2 is sample 2, etc.

(5) Using a multi-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips into the mixing wells.

(6) Using a multi-channel pipette, transfer 100 µl of each reaction mixture directly into all corresponding clear test wells. Discard the mixing wells into an appropriate waste container.

(7) Let the reaction mixture incubate for exactly 5 minutes. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

(8) At the end of the 5 minute incubation period, dump the contents of the wells into an appropriate waste container. Using a 500 ml squeeze bottle containing the wash solution, vigorously wash each well by overfilling. Repeat the vigorous wash for a total of four washes. After the last wash, invert the wells and tap on absorbent paper to remove the residual wash solution. Wipe excess liquid from the bottom of the wells.

(9) Pour the Substrate Solution into a clean reagent reservoir.

(10) Dispense 100 µl of Substrate Solution into each test well using a multi-channel pipette.

(11) Let the Substrate Solution incubate for exactly 5 minutes. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

(12) Pour the Stop Solution into a clean reagent reservoir.

(13) Dispense 100 µl of Stop Solution into each test well using a multi-channel pipette.

(14) Within 20 minutes, read and record the optical density at 650 nm using a Hyperion MicroReader™ 3 Model 4027-002 microwell reader. Make sure that the well bottoms are clean and dry before placing in reader.

b. Reading the Results.

(1) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.

(2) When prompted to "Run a test", select yes, select the appropriate test number, then press "Enter".

(3) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24) then press "Enter".

(4) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.

(5) The reader will read the optical density of the wells and print a report.

(6) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the to the instrument prompts as indicated above.

(7) Use the data reduction software provided by SDI to quantify results.

## 11.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 3.4 ppm. Results exceeding 3.4 ppm are reported as > 3.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 3.4 ppm are certified to the nearest whole ppm.

Test results over 3.4 ppm are certified as exceeding 3 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 11.6 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 6.0 ppm and the conformance limit value is 3 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 2.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 6.3 ppm (3 x 2.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 2.1 ppm
= 3 x 2.1 ppm = 6.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

## 11.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 11.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

## 11.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits.

(1) 48 antibody-coated microtiter wells (4 strips of 12) in foil pouch.

(2) 48 red-marked mixing wells in poly bag.

(3) 5 vials each containing 2 ml of: 0, 0.25, 0.5, 1.0, and 3.0 ppm of DON calibration.

(4) 1 vial containing 8 ml of DON-HRP Enzyme Conjugate.

(5) 1 vial containing 8 ml of Substrate.

(6) 1 vial containing 8 ml of Stop Solution.

(7) 1 vial containing 25 ml of 20X Wash Concentrate.

(8) 4 multi-channel pipette reservoirs.

(9) Data reduction software. (Provided separately)

b. Materials Required but not Provided.

(1) Distilled/deionized water.

(2) 100 ml graduated cyclinder.

(3) Glassware with 125 ml capacity, for sample extraction.

(4) Whatman #1 filter paper or equivalent.

(5) Filter funnel.

(6) 100 µl pipette with disposable tips.

(7) Multi-channel pipette.

(8) 500 ml plastic squeeze bottle.

(9) Hyperion MicroReader™ 3 Model 4027-002 with 650 nm filter.

(10) Timer.

(11) Blender and blender jars.

(12) Balance.

(13) Sample Grinder.

## 11.10 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

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GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
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# CHAPTER 12

## VICAM - DON FQ TEST KIT

## 12.1 TESTING AREA

The extraction solution and other materials used in the DON FQ test kit necessitates the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

## 12.2 EXTRACTION PROCEDURES

a. Preparation of Extraction Solvent (84 Percent Acetonitrile Solution).

Make up the solution by using the ratio of 84 parts acetonitrile to 16 parts deionized/distilled water. Prepare the 84 percent acetonitrile solution by adding 840 ml acetonitrile to 160 ml of distilled or deionized water. Mix well. Label the solution bottle and keep it tightly capped when not in use.

If the amount of solution being prepared needs to be adjusted based on the workload at individual locations, make sure that 84 parts acetonitrile to 16 parts deionized/distilled water ratio is maintained.

b. Extraction Procedures.

(1) Place 50 grams of ground sample into a blender jar.

(2) Add 200 ml of acetonitrile/water (84/16) and blend on high for 3 minutes.

(3) Filter into a sample container using coffee filters or Whatman No. 1 filter paper.

## 12.3 TEST PROCEDURES

a. Purification Procedures.

**NOTE: All solution transfers may be carried out using adjustable automatic pipettors with disposable tips. Care should be taken to make sure that the tips used are large enough to hold the volume being transferred. Make sure that they are securely attached to the pipettor.**

(1) Place 4 ml of extract in a 15 x 85 culture tube. Insert a MycoSep #225 column into the top of the culture tube and slowly (20 seconds) push to the bottom of the tube.

**(Note: Use 6 ml of extract and a MycoSep #227 column for malted barley samples and take 30 seconds to push the extract through the column.)**

(2) Transfer 1.5 ml of each purified sample extract to a 12 x 75 mm cuvette. Use a clean pipette tip for each transfer.

b. Calibrators and Control Preparation.

(1) Allow the calibrator and control solutions to come to room temperature.

(2) Invert each calibrator standard bottle and control standard bottle three times to mix thoroughly.

(3) Transfer 1.5 ml of the green-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(4) Using a clean tip, transfer 1.5 ml of the red-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(5) Using a clean tip, transfer 1.5 ml of the control (yellow-labeled) solution to a clean 12 x 75 mm cuvette.

(6) Cap the calibrator solutions tightly and store in the refrigerator.

(7) Proceed with the analysis, treating samples, calibrators, and control identically.

c. Evaporation Procedures.

Evaporate each sample, calibrator, and control to dryness using a vacuum manifold and dry bath set at 70°C.

**Note: To decrease the evaporation time, turn off the vacuum to the manifold for the rows that are not being used.**

d. Derivatization Procedures.

(1) Add 1.5 ml of Reagent A to all sample tubes, calibrators, and control.

(2) Add 50 microliters (µl) of Reagent B to all sample tubes, calibrators, and control. Cap the tubes and mix contents with a vortex for 10 seconds.

(3) Heat the tubes in a 50°C bath for 10 minutes.

(4) Remove tubes from the bath and cool to room temperature. Read the samples in the fluorometer within 1 hour.

**Note: Cuvettes may be placed in tap water for 30 seconds to cool. Dry the outside wall of the cuvette completely before placing in the fluorometer.**

e. Fluorometer Reading.

Calibrate the fluorometer using the following procedure:

(1) Turn on the power (no warm-up is necessary).

(2) Change the date or time - If correct, press the "Continue" key.

(3) When asked for Test Delay Time, enter "2" and press the "Enter" key.

(4) When asked for answer format, select "Decimals."

(5) When asked for measurement units, select "ppm."

(6) At the "insert red vial" prompt, place the appropriate calibrator cuvette into the sample well.

(7) When asked for the calibrator value, enter the appropriate value (refer to the card supplied with the calibrators for the red value) and press the "Enter" key.

(8) When asked to "remove the red vial," remove the calibrator tube from the sample well.

(9) At the "insert green vial" prompt, place the appropriate calibrator cuvette into the sample well.

(10) When asked for the "blank value, "enter the appropriate value (refer to the card supplied with the calibrators for the green value) and press the "Enter" key.

(11) When asked to "remove the green vial," remove the calibrator tube from the sample well.

(12) At the "insert test vial" prompt, place the control cuvette into the sample well.

(13) The fluorometer will now display the value for the control vial.

(14) Compare the value of the control with the values listed on the card. If the control value is within the specified range, the fluorometer is ready to analyze samples. If the value is outside of the specified range, rerun the red, green, and yellow calibration cuvettes. If the control value still exceeds the specified range limit, contact Vicam.

(15) Press the "Enter" key. The fluorometer is now ready to analyze samples.

f. Reading the Results.

To determine the DON concentration insert the cuvette containing the sample portion into the sample well of the fluorometer. The DON concentration will appear on the display after the appropriate 2-second delay. Read the results.

## 12.4 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

## 12.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (acetonitrile/water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

## 12.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

## 12.7 WASTE DISPOSAL

Transfer sample extract solutions (acetonitrile/water) and derivatization solutions into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Transfer sample slurry, used filter paper, cuvettes, caps, and columns into the normal solid waste container for routine disposal.

## 12.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) Glass culture tubes (15 x 85 mm) - 25 tubes per test kit

(2) DON FQ (or DON FQ MB for malted barley) columns

(3) Glass cuvettes and caps (12 x 75 mm) - 50 per test kit

(4) Reagent A - Ethylenediamine in methanol

(5) Reagent B - Zirconyl Nitrate in methanol

(6) Calibrator solutions (red-label, and green-label) plus control solution (yellow-label)

b. Materials Required but not Provided:

(1) Blender and blender jars. The unit must be explosion proof.

(2) Funnel

(3) Beakers - 250 ml

(4) 250 ml graduated cylinder

(5) Extraction Solvent - Acetonitrile/distilled or deionized water (84/16)

(6) Filter paper - Vicam part # 31240, Whatman No. 1, or equivalent

(7) Carboy - 2 gallon capacity

(8) Vortex mixer

(9) Pipette and tips - 50 µl

(10) Pipette and tips - 1.5 ml

(11) Fluorometer with printer - Romer RL 100, Vicam Series III and IV, Torbex 100, Vicam FX-100)

(12) Vacuum Pump and Trap

(13) E-Vap Evaporator

(14) Test Tube Rack

(15) Thermometer

(16) Dry Bath with Heating Block

(17) Sample grinder

(18) Balance

## 12.9 STORAGE CONDITIONS

a. Columns.

DON FQ and DON FQ MB columns - Room temperature in a drawer or box.

b. Reagents.

Reagents A & B are shipped in amber bottles, cap tightly and store in a temperature controlled area (between 40° and 80° F). Do not freeze. Reagents should stay stable for 6 months.

c. Calibration and Control Solutions.

Calibration and control solutions are shipped in amber vials, cap tightly and store in the refrigerator. Solutions should stay stable for 6 months.